#4

N-TERMINAL AMINOACID SEQUENCES

Position	A	B	C
01			LEU
02			ALA
03			VAL
04			PRO
05		ALA	ALA
06		SER	SER
07		---	ARG
08	---**	---	ASN
09	GLN	GLN	GLN
10	SER	SER	SER
11	SER	SER	SER
12	---	---	GLY
13	ASP	ASP	ASP
14	THR	THR	THR
15	VAL	VAL	VAL
16	ASP	ASP	ASP
17	GLN	GLN	
18		GLY	
19		TYR	
20		GLN	
21		ARG	
22		PHE	
23		SER	
24		GLU	
25		THR	
26		SER	
27		HIS	
28		LEU	
29		ARG	
30		(GLY)*	
31		GLN	
32		TYR	
33		ALA	
34		PRO	
35		PHE	
36		PHE	
37		(ASP)	
38		LEU	
39		ALA	

FIG. 1 A

PEPTIDE AMINOACID SEQUENCES

Position	A	B	C	D	E
01	GLN	(TRP)*	MET	ALA	VAL
02	———**	SER	MET	SER	VAL
03	GLN	PHE	GLN	SER	ASP
04	ALA	ASP	CYS	ALA	———
05	GLU	THR	GLN	GLU	ARG
06	GLN	ILE	ALA	LYS	PHE
07	GLU	SER	GLU	GLY	PRO
08	PRO	THR	GLN	TYR	TYR
09	LEU	SER	GLU	ASP	THR
10	VAL	THR	PRO	LEU	GLY
11	(ARG)	VAL	LEU	VAL	———
12	VAL	ASP	VAL	VAL	ALA
13	LEU	THR	ARG		
14	VAL	LYS	VAL		
15	ASN	LEU	LEU		
16	(ASP)	SER	VAL		
17	(ARG)	PRO	ASN		
18	(VAL)	PHE	ASP		
19	VAL	(CYS)	ARG		
20	PRO	(ASP)			
21		LEU			
22		PHE			
23		THR			

FIG. 1B

N-TERMINUS 100KD PROTEIN

Position	
01	VAL
02	VAL
03	ASP
04	GLU
05	ARG
06	PHE
07	PRO
08	TYR
09	THR
10	GLY

FIG. 1C

```
             1   2   3   4   5   6   7   8   9   10  11  12  13  14  15  16  17  18  19  20
Peptide C:  Leu-Ala-Val-Pro-Ala-Ser-Arg-Asn-Gln-Ser-Ser-Gly-Asp-Thr-Val-Asp
Peptide B:                  Ala-Ser-***-***-Gln-Ser-Ser-***-Asp-Thr-Val Asp-Gln-Gly-Tyr-Gln-
Peptide A:                              ***-Gln-Ser-Ser-***-Asp-Thr-Val-Asp-Gln

Possible
codons:5'   CTG-GCG-GTG-CCG-GCG-TCG-CGG-AAT-CAA-TCG-TCG-GGG-GAT-ACG-GTG-GAT-CAA-GGG-TAT-CAA-
              A   A   A   A   A   A   A   C   G   A   A   A   C   A   A   C   G   A   C   G
              T   T   T   T   T   T   T           T   T   T       T   T           T
              C   C   C   C   C   C   C           C   C   C       C   C           C
             TTA                 AGT AGA         AGT AGT
              G                   C   G           C   C

AB1024:      3'-CGG-CAG-GGG-CGG-TCG-GCG-TTG-GTC-TCG-TCG-CCG-CTG-TGG-CAG-CTG-GTC

AB1065:                                              3'-CCG-CTG-TGG-CAC-CTG-GTC
                                                                              A
AB1066:                                                                   G
AB1067:                                                               A
AB1068:                                                           A
AB1069:                                                       A
AB1070:                                                       A   A   A   G   A

AB1226:                                                          3'-CAG-CTG-GTC-CCG-ATG-GTC
                                                                      C   A       A

AB1227:                                                          3'-CAG-CTG-GTC-CCG-ATG-GTC
                                                                      C   A   T   A   A   T
                                                                      A           T

AB1298:                                                  3'-CTG-TGG-CAG-CTG-GTG-CCG-ATG-GTC
                                                              A   C   C   A   T   C   A   T
```

FIG.2A-I

(phytase N–terminus, continued)

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Peptide B:	(Arg)–	Phe–	Ser–	Glu–	Thr–	Ser–	His–	Leu–	Arg–	(Gly)–	Gln–	Tyr–	Ala–	Pro–	Phe–	Phe–	(Asp)–	Leu–	Ala
	CGG-	TTT-	TCG-	GAG-	ACG-	TCG-	CAT-	CTG-	CGG-	GGG-	CAG-	TAT-	CGC-	CCG-	TTT-	TTT-	GAT-	CTG-	GCG
	T	A	C	A	A	A	A	C	A	A	A	C	C	A	C	C	C	A	A
	T		T		T	T		T	T	T			T	T				T	T
	C		C		C	C		C	C	C			C	C				C	C
	AGG		AGT			AGT		TTG	AGG									TTG	
	A		C			C		A	A									A	
AB1388:										3'-CCG-	GTC-	ATG-	CGG-	GGG-	AAG-	AAG-	CTG-	GA	
										C			C	C				A	

FIG.2A–2

```
            1    2    3    4    5    6    7    8    9    10    11           12   13   14   15
Peptide A: (Gln- ? -Gln-Ala-Glu-Gln-Glu-Pro-Leu-Val-(Ser/Arg)-Val-Leu-Val-(Asp/Asn)

            CAG-???-CAG-GCG-GAG-CAG-GAG-CCG-CTG-GTG-(TCG/CGG)-GTG-CTG-GTG-(GAT/AAT)
              A      A   A   A   A   A   A   A   A   A   A   A   A   A   C   C
                             T               T   T   T   T   T   T   T   T
                             C               C   C   C   C   C   C   C   C
                                           TTG     AGT AGG     TTG
                                             A       C           A

AB1295:          3'-GTC.CGC.CTC.GTC. CTC. GGG. GAG. CA-5'
                      T   G   T   T    T    C A  C


           16             17        18  19    20   21   22
          -Asp/Thr/Arg-(Arg/Val)-Val-Pro-(Pro)-Met-Gly

          -GAT/ACG/CGG-(CGG/GTG)-GTG-CCG-(CCG)-ATG-GGG
           C   A   A    A   A   A   A   A       A
                   T    T   T   T   T   T       T
                   C    C   C   C   C   C       C
                 AGG  AGG
                   A    A
```

FIG.2B-1

```
            1    2   3   4   5   6  7   8    9  10  11  12  13  14  15  16  17  18
Peptide B: (Trp)-Ser-Phe-Asp-Thr-Ile-Ser-Thr- Ser-Thr-Val-Asp-Thr-Lys-Leu-Ser-Pro-Phe-

           (TGG)-TCG-TTT-GAT-ACG-ATA-TCG-ACG-TCG-ACG-GTG-GAT-ACG-AAG-CTG-TCG-CCG-TTT-
                   A   C   C   A   T   A   A   A   A   A   C   A   A   A   A   A   C
                   T           T   C   T   T   T   T   T       T       T   T   T
                   C           C       C   C   C   C   C       C       C   C   C
                 AGT                 AGT     AGT                     TTG AGT
                   C                   C       C                       A   C

AB1296 :        3'-AAG. CTG.TGC. TAG.AGG. TGG.AGG.TGG. CAC. CTG. TGC. TTC-5'
                                     TCC     C TCC    C
                                                              AB1297:  3'-GGC.AAG.
                                                                              G

19     20    21  22  23    24    25    26    27    28   29         30    31       32   33
(Cys)-(Asp)-Leu-Phe-Thr-(Thr)-(Asp)-(Glu)-(Cys)-(Ile)-(Thr/Asn)-(Tyr)-(Arg/Gly)-(Tyr)-Leu

(GTG)-(GAT)- CTG- TTT- ACG-(ACG)-(GAT)- GAG) - (TGT)-(ATA)-(ACG/AAT) - (TAT)-(CGG/GGG)-(TAT)-CTG
  C     C     A    C    A    A     C     A      C     T     A   C      C     A     A    C    A
              T         T    T                        C     T                T     T         T
              C         C    C                              C                C     C         C
             TTG                                                            AGG             TTG
              A                                                              A               A

(ACG). (CTG). GAG.  AAG. TGC. (TGC). (CTG). (CTC).  (ACG).(TAG). (T)-5'
               C          G     G
```

FIG.2B-2

```
1     2     3     4     5     6     7     8     9     10    11    12    13    14    15    16    17    18    19    20    21    22    23
Phe-Ser-Tyr-Gly-Ala-Ala-Ile-Pro-Gln-Ser-Thr-Gln-Glu-Lys-Gln-Phe-Ser-Gln-Glu-Phe-Arg-Asp-Gly

5'-TTT-TCG-TAT-GGG-GCG-GCG ATA-CCG-CAG-TCG-ACG-CAG-GAG-AAG-CAG-TTT-TCG-CAG-GAG-TTT-CGG-GAT- GGG
     C   A   C   A   A   A   T   A   A   A   A   A   A   A   A   C   A   A   A   C   A   C   A
         T       T   T   T   C   T       T   T                       T               T       T
         C       C   C   C       C       C   C                       C               C       C
       AGT                             AGT                         AGT             AGG
         C                               C                           C               C

AB1025: 3'-ATG-CCG-CGG-CGG-TAG-GGG-GTC-TCG-TGG-GTC-CTC-TTC-GTC-AAG-TCG-GTC-CTC-AAG-GC-5
AB1026:                                    3'-GTC-CTC-TTC-GTC-AAG-TCG-GTC-CTC-AAG-GC-5
                                                    T                AGA         T
                                                                       C

AB1027: 3'-ATG-CCG-GCG-CGC-TAA-GGC-GTC-5'
                 A   T   T   G   G
                     A   A
                     G   G
```

FIG.3

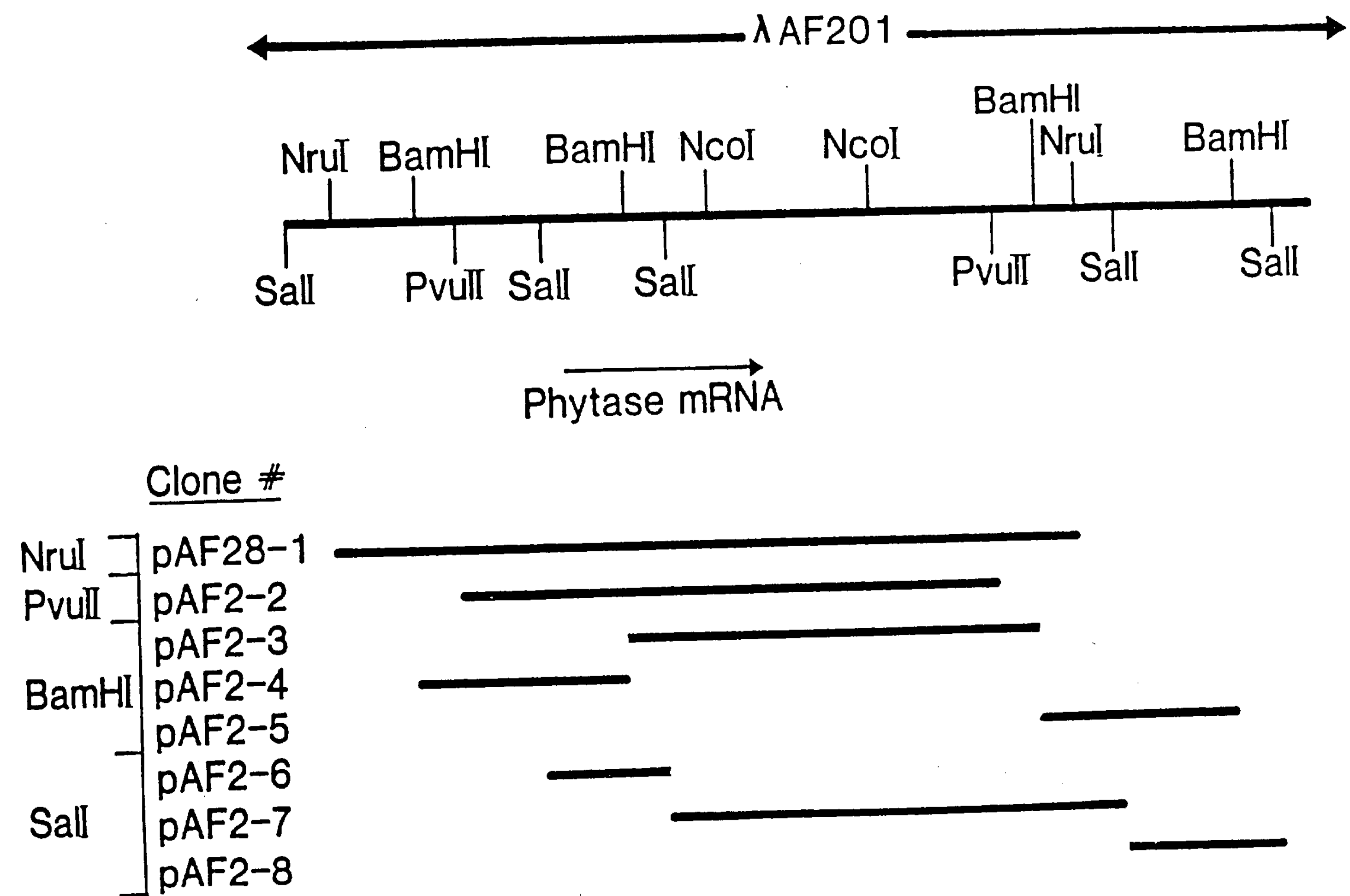
λ AF201
NruI
BamHI
BamHI
NcoI
NcoI
BamHI
NruI
BamHI
SalI
PvuII
SalI
SalI
PvuII
SalI
SalI
Phytase mRNA
Clone #
NruI
PvuII
BamHI
SalI
pAF28-1
pAF2-2
pAF2-3
pAF2-4
pAF2-5
pAF2-6
pAF2-7
pAF2-8

FIG.4

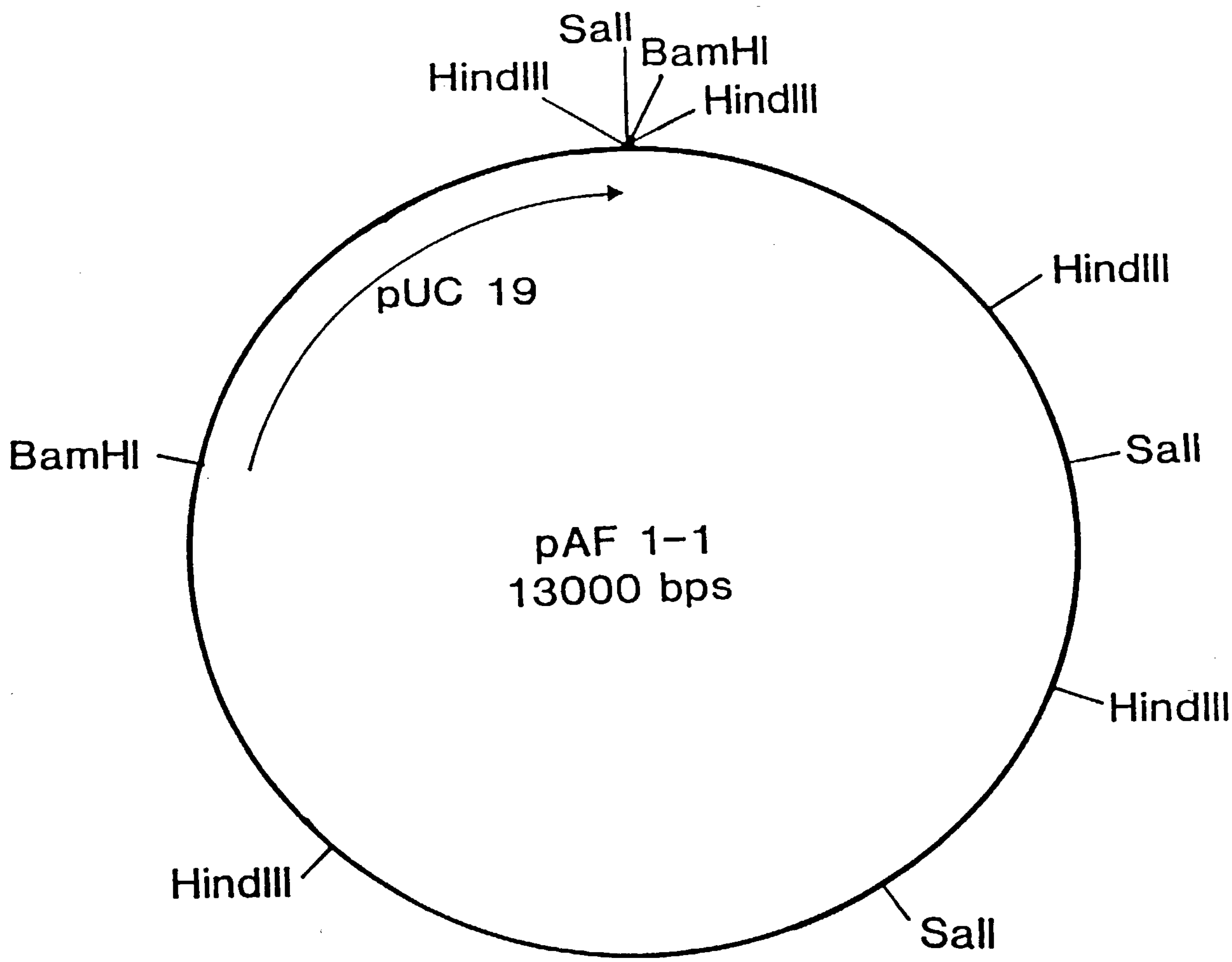
SalI
BamHI
HindIII
HindIII
HindIII
SalI
HindIII
SalI
HindIII
BamHI
pUC 19
pAF 1-1
13000 bps

FIG.5

```
GTCGACTTCCCGTCCTATTCGGCCTCGTCCGCTGAAGATCCATCCCACCA
 SalI

TTGCACGTGGGCCACCTTTGTGAGCTTCTAACCTGAACTGGTAGAGTATC    100

ACACACCATGCCAAGGTGGGATGAAGGGGTTATATGAGACCGTCCGGTCC

GGCGCGATGGCCGTAGCTGCCACTCGCTGCTGTGCAAGAAATTACTTCTC    200

ATAGGCATCATGGGCGTCTCTGCTGTTCTACTTCCTTTGTATCTCCTGTC
         translation start

TGGGTATGCTAAGCACCACAATCAAAGTCTAATAAGGACCCTCCCTTCCG    300
   start<-------------------------------------------

AGGGCCCCTGAAGCTCGGACTGTGTGGGACTACTGATCGCTGACTATCTG
--intron------------------------------------------

TGCAGAGTCACCTCCGGACTGGCAGTCCCCGCCTCGAGAAATCAATCCAG    400
->end

TTGCGATACGGTCGATCAGGGGTATCAATGCTTCTCCGAGACTTCGCATC

TTTGGGGTCAATACGCACCGTTCTTCTCTCTGGCAAACGAATCGGTCATC    500

TCCCCTGAGGTGCCCGCCGGATGCAGAGTCACTTTCGCTCAGGTCCTCTC

CCGTCATGGAGCGCGGTATCCGACCGACTCCAAGGGCAAGAAATACTCCG    600

CTCTCATTGAGGAGATCCAGCAGAACGCGACCACCTTTGACGGAAAATAT

GCCTTCCTGAAGACATACAACTACAGCTTGGGTGCAGATGACCTGACTCC    700

CTTCGGAGAACAGGAGCTAGTCAACTCCGGCATCAAGTTCTACCAGCGGT

ACGAATCGCTCACAAGGAACATCGTTCCATTCATCCGATCCTCTGGCTCC    800

AGCCGCGTGATCGCCTCCGGCAAGAAATTCATCGAGGGCTTCCAGAGCAC

CAAGCTGAAGGATCCTCGTGCCCAGCCCGGCCAATCGTCGCCCAAGATCG    900
         BamHI

ACGTGGTCATTTCCGAGGCCAGCTCATCCAACAACACTCTCGACCCAGGC

ACCTGCACTGTCTTCGAAGACAGCGAATTGGCCGATACCGTCGAAGCCAA   1000
```

FIG.6A

TTTCACCGCCACGTTCGTCCCCTCCATTCGTCAACGTCTGGAGAACGACC

TGTCCGGTGTGACTCTCACAGACACAGAAGTGACCTACCTCATGGACATG 1100

TGCTCCTTCGACACCATCTCCACCAGCACCGTCGACACCAAGCTGTCCCC
SalI

CTTCTGTGACCTGTTCACCCATGACGAATGGATCAACTACGACTACCTCC 1200

AGTCCTTGAAAAAGTATTACGGCCATGGTGCAGGTAACCCGCTCGGCCCG

ACCCAGGGCGTCGGCTACGCTAACGAGCTCATCGCCCGTCTGACCCACTC 1300

GCCTGTCCACGATGACACCAGTTCCAACCACACTTTGGACTCGAGCCCGG

CTACCTTTCCGCTCAACTCTACTCTCTACGCGGACTTTTCGCATGACAAC 1400

GGCATCATCTCCATTCTCTTTGCTTTAGGTCTGTACAACGGCACTAAGCC

GCTATCTACCACGACCGTGGAGAATATCACCCAGACAGATGGATTCTCGT 1500

CTGCTTGGACGGTTCCGTTTGCTTCGCGTTTGTACGTCGAGATGATGCAG

TGTCAGGCGGAGCAGGAGCCGCTGGTCCGTGTCTTGGTTAATGATCGCGT 1600

TGTCCCGCTGCATGGGTGTCCGGTTGATGCTTTGGGGAGATGTACCCGGG

ATAGCTTTGTGAGGGGGTTGAGCTTTGCTAGATCTGGGGGTGATTGGGCG 1700

GAGTGTTTTGCTTAGCTGAATTACCTTGATGAATGGTATGTATCACATTG
translation stop

CATATCATTAGCACTTCAGGTATGTATTATCGAAGATGTATATCGAAAGG 1800

ATCAATGGTGACTGTCACTGGTTATCTGAATATCCCTCTATACCTCGTCC

CACAACCAATCATCACCCTTTAAACAATCACACTCAACGCACAGCGTACA 1900

AACGAACAAACGCACAAAGAATATTTTACACTCCTCCCCAACGCAATACC

AACCGCAATTCATCATACCTCATATAAATACAATACAATACAATACATCC 2000

FIG.6B

ATCCCTACCCTCAAGTCCACCCATCCTATAATCAATCCCTACTTACTTAC

TTCTCCCCCTCCCCCTCACCCTTCCCAGAACTCACCCCCGAAGTAGTAAT 2100

AGTAGTAGTAGAAGAAGCAGACGACCTCTCCACCAATCTCTTCGGCCTCT

TATCCCCATACGCTACACAAAACCCCCACCCCGTTAGCATGCACTCAGAA 2200

AATAATCAAAAATAACTAAGAAGGAAAAAAAAGAAGAAGAAAGGTTACAT

ACTCCTCTCATACAAACTCCAAGACGTATACATCAAGATGGGCAATCCCA 2300

CCATTACTGATATCCATCTATGAACCCATTCCCATCCCACGTTAGTTGAT

TACTTTACTTAGAAGAAGAAAAAGGGAAGGGAAGGGAAAGAAGTGGATGG 2400

GATTGAGTTAGTGCTCACCGTCTCGCAGCAAGTTTATATTCTTTTGTTTG

GCGGATATCTTTCACTGCTCCTGCTGGACGTTGTCACGGGGTGGTAGTGG 2500

TTGGCGGTGGTGAGGGTCCATGATCACTCTTGGTTTGGGGGGTTGTTGTT

GTCGTTGTTGTTGTTGTTGGGTGGGCATTTTCTTTTCTTCACTTGGGGAT 2600

TATTATTTGGAATTGGTTAGTTTGAGTGAGTGGGTAATATTGAATGGGTG

ATTATTGGGAATGAAGTAGATTTGGCTATGAATGGTTGATGGGATGGAAT 2700

GAATGGATGGATGAATAGATGGAGGCGGAAAAGTCAGGTGGTTTGAGGTT

CGGATTATTATCTTTGTGCCTGAGGCATCACTCTCCATCTATGTTGTTCT 2800

TTCTATACCGATCTACCAGAGCTAAGTTGACTGATTCTACCACAGTGCAC

AATAAGTATGTACTTATTTCATTTAGAGTATTTAGATTAACCCGCTGTGC 2900

TATTTGCCGTAGCTTTCCACCCAATTTCGAAGTTCGAAGAATTAAAACTC

ATCCTACAGTACAGAATAGAAGTAAAAGGAGAAGAGAAAAACAAGATAAT 3000

FIG.6C

ACAACCAGTCCAGGTCCATTCTAGATCTCGAATGACCACCAAATAAGAAA

GCAACAAGCAAGTAAGCAAAGCATAAGTCTAAATGAACGCCAATAACTTC 3100

ATCGCCTGCCTTTGAAACTGAACGCTATGCACGAATGGCTCGAAATGATT

CCCTTAACTCCGTAGTATTGAGAGTGAGAGGAAAAGAAAAAAAGAGACAG 3200

AAAAGCTGACCATGGGAAAGAAGCATGATCAGTCGGGAATGGATCTGCGG

GTTGAGATAGATATGAGTTGCCTCGCAGATCCGGTGACAAGATAAGAGAA 3300

TTGGGAGATGTGATCAGCCACTGTAACTTCATCAAGCATCGACATTCAAC

GGTCGGGTCTGCGGGTTGAGATGCAAGTTGAGATGCCACGCAGACCCGAA 3400

CAGAGTGAGAGATGTGAGACTTTTGAACCACTGTGACTTCATCAAGCATC

AAAACACACTCCATGGTCAATCGGTTAGGGTGTGAGGGTTGATATGCCAG 3500

GTTCGATGCCACGCAGACCCGAACCGACTGAGAAATATGAAAAGTTGGAC

AGCCACTTCATCTTCATCAAGCGTAAAACCCCAATCAATGGTAAATCGAA 3600

AACGAATCTGCGGGCTGATGTGGAAATGAGACGAATGCCTCGCAGATTCG

AAGACACGTAAATCGAGATGAACAATCACTTTAACTTCATCAAAGCCTTA 3700

AATCACCCAATGGCCAGTCTATTCGGGTCTGCGGGTTGAGGTTCCTGTTG

AGATGCCACGCAGACTGCGAACATGCGATGCATTATAAGTTGGACGAGTG 3800

TAGACTGACCATTGATAACCGAGATAAACAATCACTTCAACTTCATCAAA

GCCTTAAATCACTCAATGGCCAGTCTGTTTGCGGTCTGCGGGCTGATACC 3900

CAAGTTGCGATGCCACGCAGACTGCAAACATTGATCGAGAGACGAGAAAA

ACAACGCACTTTAACTTCAACAAAAGCCTTTCAATCAGTCAATGGCCAGT 4000

FIG.6D

CTGTTCGCGGTCTGCGGGCTGATATGCGAGTTGAGGTGCCTCGCAGACCG

CGAACATGCGATGTAATTTCTTAGTTAGACGAGTGCCTGGCCATTGAGAA 4100

ACGAGAGAAACAACCACTTTAACTTCATGAAAGCCTTGAACTACTCAATG

ACCCGTCTGTTGGCGGTCTGCGGGCTGATATTCGAGTTGAGATGCCACGC 4200

AGACCGCCAACATGCGATGTATCATGTAAGTTAGATGAGTGACTGGCCAT

TGAGAAACGAGAGAAACAACCACACTTCATGAGAGCCTTAAATTATTCAA 4300

TGACCAGTCTGTTCACGGTCTGCGGGTTGGTATGCGAGTCGAGGTGCCTC

GCAGACCGCGAACATGCGATGTTTTCGATGGACGAGTGAAGCCTGACGAT 4400

CGAGAACTATCTCAGTTGGGTTGGCCATTCGGCTGGCCGTTGGGTTTAGT

ATTAGGATCGTCAGGTTTGTCCGATGGAACGTTCCGTTTGCGTGCGTTGG 4500

CGCGACGAGCCCTCTCCTCGGCGTGATTCTGAAATTCTGCAATCAGGGCA

GCCGCAGCACGGCGACGGGACGTCCTCCAGGAGCTGTGTTGAAGTTTCGG 4600

GGTGGCGGTCCAGAAGGGGGAGTTACATTAAAAGCCTCATAGATGTCTTT

GGGTGGTTCCGGGGGGCCCATCGCAAGATCTTCTGGAGTTGTGCGTCTGA 4700

TCATCTCTTGAGTGTAATTGCGACGCAGACCGAGCTTCAGGATTTTGGAA

GGGCTGGATCGCTCCTGCTGACTCTTTCCCTCAGCGGGCTTCGTCTCGGC 4800

AGTCTTCATTTCGGCGGGCTGATCTTCCATCTCAGAATGGGATCGCTTTC

TGGTCGCTGCACCCGCTCCTCCCTTCAAGGTCAGCTTGATGCGCAGCGTC 4900

TTGGGCGGCTCAGCTGGTGGAGTTGGTTCCGGCTCTGGCTCCCTCCGGCG

TCGCTTGGGCACTTGAGTAGTCTCTGAGGCTTCGCCGCGGCGCCGTTTGC 5000

GAGTCGGCTCCTTGGTCTCTTTGGCCTCTTTCACTTCACCTGGACCGTCT

TTCGGGGCGGTTTCATCGTGCTGAGCGATCAAGGTTTGGATGTAGGCAGC 5100

CGGCATCATTCGATCAACGGCAATTCCTCTCTTGCGGGCCTCCTCCCGAG

CCTTGATTGTCGCCTTGACCTCGTCCACGTTTTCGAAGAAGAAAGGCATC 5200

TTGTTATCCTGAGGCAAGTTGCGCTCTCCCATGCGTGGGGATATCCGAAG

ATGCGGTCCTTCTCGAACTGTTCATGAGACTTCAGACGAATTGGAGGCTG 5300

GGGGAGCAATTTGTCTCCGTAGGTGTTGTTAGGGCGGAACCAAGAATAGC

CTTCGCCTACAACGACAAGCTCTTCGCCAAATTTATTTTTTTGGCCTGTA 5400

AAAACGAACCCATCCTCGTCAGTCCACCGGTGCGTCTCGGACGTAGAGAT

TGGCTTACTTATTCCCTCAACGCCGATCTCTGCCTGGGGCTGCGCTTCGG 5500

ATGCGGCCTCGGTCACGGCTCCGCCTCGGACTGCACCGCTGGAGTTTCGG

TCTTCTTCTCCTGCTTCTCCAGGTACTCCTTGCGTAACTCTTCGATCAGC 5600

CTCGGCTTCCGATGACTGCTCAAATTCTGGAGCAACAGCTGCCGCGGCCA

GGTCAAGCAGGCGGTTTGCTAAAACTGCCCATTTTCCATCGACACCTGCC 5700

TCCGACGCCTGTGCAAAACCAGCTGTTTTCGCATTGGCCTGTTTGTTGGC

ACGCGTCTTCTTGACTGCTGCCTTGCCCTTTACTTCCTTGAGAGCAGACT 5800

CTGGCTTAGATGATGGTGCACGGTTTCTGCGGAAGCGCCGCTCAGATTCC

AAAGATTCCATAGCTTTAATGGTAGGCTTTCTGGTTCTTCCAGAAGTGCG 5900

CGCAGCTGACGTAGTGGTTGAGTAGCTGGCAGTTGGGGATCCTGGGCCCT

CATTGGAACCATCAAGACCAAATTTGTTTCCATACATATCAGCATGGTAT 6000

FIG.6F

TCAAAAGGAAAACTTTCGCCGTACGGAGTACTGCGTTCGATTCCGGGTGT

ATCCAAGTCGTATCCAGACATGGTGTCGAATTCAGCCTTGCTGTCAAGAG 6100

CAGGGGTACTTTCAATGCTGTCAGCAACCACGCGGCCAAAGGGCGTCTTC

GGGAAAGAAGGTGTTTCAAGAGAAGCGTCATCCACGGCCTGGCTTGCGGC 6200

GTTGATTGCAGACTTTCGAGTAGATCGCTGAGGTCGCGAACTGGTTCGAG

TAGCAACCTGTGAATTGGCAGCCTTGTGACTGCTTCGATTCACTGCAGAG 6300

ACGGAGTAGACTGCACTGATTTGGAATTCTGAGTCGCAGCCATTCTGGAT

TTGCGTTCGGCGCGACGAGATCTCGCAGTCGTGGTACGAGGAGTAGAGCG 6400

AGGCTGCGTAGCAGTGTTGCAAGCTTGGTGCTAGCCTCCTGGGCTTCAGC

AGCTTCAGCAGTGGTGGCAGACGCAGCAGAATTAGCGGAGCTTTATCGGC 6500

TTTGCCGCTCTGAGCGTTGGGAGTAGAAGTGAGAGAAGAGGTAGAGTCCA

CGGAAGAAGTCTTCTCGCTGTTCTCAAAGCCGTTCAGCTTTGCTGGCATA 6600

GACTTACGCGTCTTGCGGCTGTTGGAAGCGGAAGAGTTCATGGCGGGAGA

GGAGACGTTAGAAGTAGACATGGTGGGGTTTGTTGACGGGTTTTGAGTAA 6700

CAAGAGACTTGCGTCGATCTTTGAGTGTTCTTGACAGAAAGTTATGCAAC

<u>GTCGAC</u> 6756
SalI

FIG.6G

PHYTASE LOCUS

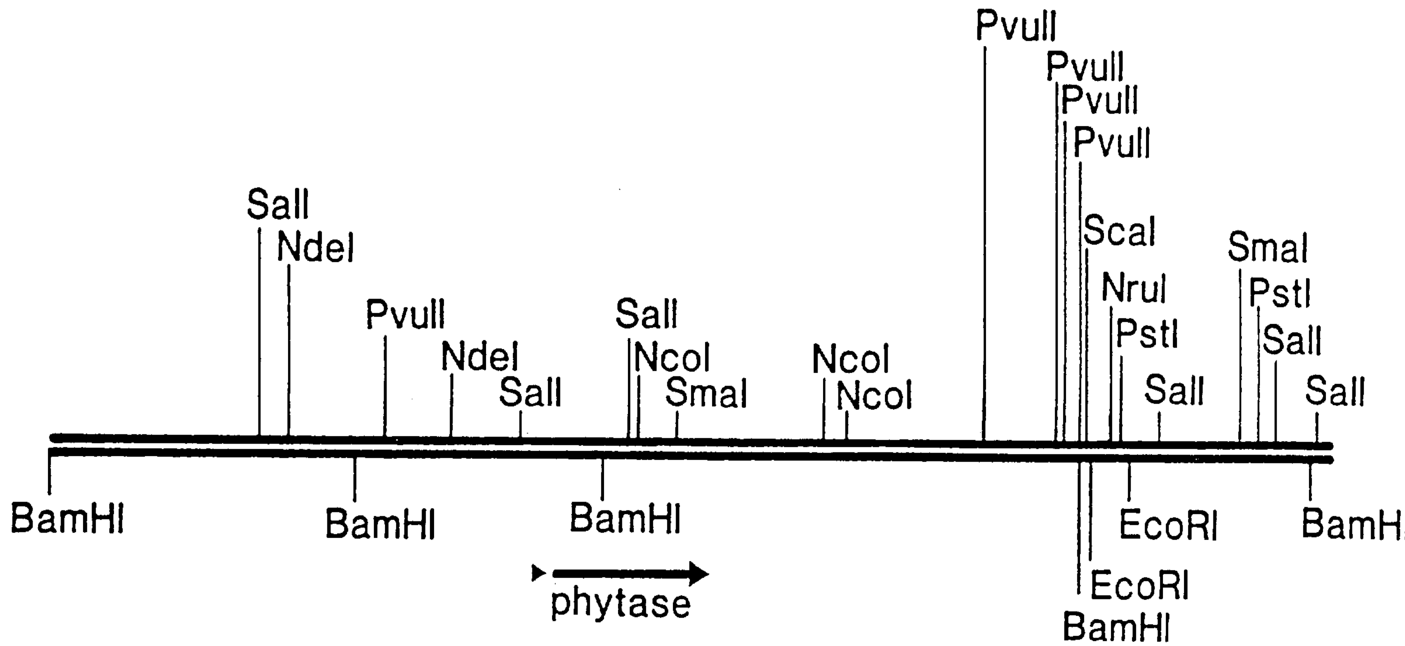

FIG.7

```
ATGGGCGTCTCTGCTGTTCTACTTCCTTTGTATCTCCTGTCTGGAGTCAC
 M  G  V  S  A  V  L  L  P  L  Y  L  L  S  G  V  T
-23    -20              '                 -10

CTCCGGACTGGCAGTCCCCGCCTCGAGAAATCAATCCAGTTGCGATACGG      100
  S  G  L  A  V  P  A  S  R  N  Q  S  S  C  D  T
     '          -1 +1            '             10

TCGATCAGGGGTATCAATGCTTCTCCGAGACTTCGCATCTTTGGGGTCAA
V  D  Q  G  Y  Q  C  F  S  E  T  S  H  L  W  G  Q
            '             20                '

TACGCACCGTTCTTCTCTCTGGCAAACGAATCGGTCATCTCCCCTGAGGT      200
 Y  A  P  F  F  S  L  A  N  E  S  V  I  S  P  E  V
    30                '           40

GCCCGCCGGATGCAGAGTCACTTTCGCTCAGGTCCTCTCCCGTCATGGAG
  P  A  G  C  R  V  T  F  A  Q  V  L  S  R  H  G
  '            50              '               60

CGCGGTATCCGACCGACTCCAAGGGCAAGAAATACTCCGCTCTCATTGAG      300
A  R  Y  P  T  D  S  K  G  K  K  Y  S  A  L  I  E
            '             70                '

GAGATCCAGCAGAACGCGACCACCTTTGACGGAAAATATGCCTTCCTGAA
 E  I  Q  Q  N  A  T  T  F  D  G  K  Y  A  F  L  K
    80                '           90

GACATACAACTACAGCTTGGGTGCAGATGACCTGACTCCCTTCGGAGAAC      400
  T  Y  N  Y  S  L  G  A  D  D  L  T  P  F  G  E
  '           100              '              110

AGGAGCTAGTCAACTCCGGCATCAAGTTCTACCAGCGGTACGAATCGCTC
Q  E  L  V  N  S  G  I  K  F  Y  Q  R  Y  E  S  L
            '            120                '

ACAAGGAACATCGTTCCATTCATCCGATCCTCTGGCTCCAGCCGCGTGAT      500
 T  R  N  I  V  P  F  I  R  S  S  G  S  S  R  V  I
   130                '          140

CGCCTCCGGCAAGAAATTCATCGAGGGCTTCCAGAGCACCAAGCTGAAGG
  A  S  G  K  K  F  I  E  G  F  Q  S  T  K  L  K
  '           150              '              160

ATCCTCGTGCCCAGCCCGGCCAATCGTCGCCCAAGATCGACGTGGTCATT      600
D  P  R  A  Q  P  G  Q  S  S  P  K  I  D  V  V  I
            '            170                '

TCCGAGGCCAGCTCATCCAACAACACTCTCGACCCAGGCACCTGCACTGT
 S  E  A  S  S  S  N  N  T  L  D  P  G  T  C  T  V
   180                '          190

CTTCGAAGACAGCGAATTGGCCGATACCGTCGAAGCCAATTTCACCGCCA      700
  F  E  D  S  E  L  A  D  T  V  E  A  N  F  T  A
  '           200              '              210
```

FIG.8A

```
CGTTCGTCCCCTCCATTCGTCAACGTCTGGAGAACGACCTGTCCGGTGTG
T  F  V  P  S  I  R  Q  R  L  E  N  D  L  S  G  V
            '           220              '

ACTCTCACAGACACAGAAGTGACCTACCTCATGGACATGTGCTCCTTCGA      800
 T  L  T  D  T  E  V  T  Y  L  M  D  M  C  S  F  D
  230               '              240

CACCATCTCCACCAGCACCGTCGACACCAAGCTGTCCCCCTTCTGTGACC
  T  I  S  T  S  T  V  D  T  K  L  S  P  F  C  D
  '         250              '              260

TGTTCACCCATGACGAATGGATCAACTACGACTACCTCCAGTCCTTGAAA      900
L  F  T  H  D  E  W  I  N  Y  D  Y  L  Q  S  L  K
            '           270              '

AAGTATTACGGCCATGGTGCAGGTAACCCGCTCGGCCCGACCCAGGGCGT
 K  Y  Y  G  H  G  A  G  N  P  L  G  P  T  Q  G  V
  280               '              290

CGGCTACGCTAACGAGCTCATCGCCCGTCTGACCCACTCGCCTGTCCACG      1000
  G  Y  A  N  E  L  I  A  R  L  T  H  S  P  V  H
  '         300              '              310

ATGACACCAGTTCCAACCACACTTTGGACTCGAGCCCGGCTACCTTTCCG
D  D  T  S  S  N  H  T  L  D  S  S  P  A  T  F  P
            '           320              '

CTCAACTCTACTCTCTACGCGGACTTTTCGCATGACAACGGCATCATCTC      1100
 L  N  S  T  L  Y  A  D  F  S  H  D  N  G  I  I  S
  330               '              340

CATTCTCTTTGCTTTAGGTCTGTACAACGGCACTAAGCCGCTATCTACCA
  I  L  F  A  L  G  L  Y  N  G  T  K  P  L  S  T
  '         350              '              360

CGACCGTGGAGAATATCACCCAGACAGATGGATTCTCGTCTGCTTGGACG      1200
T  T  V  E  N  I  T  Q  T  D  G  F  S  S  A  W  T
            '           370              '

GTTCCGTTTGCTTCGCGTTTGTACGTCGAGATGATGCAGTGTCAGGCGGA
 V  P  F  A  S  R  L  Y  V  E  M  M  Q  C  Q  A  E
  380               '              390

GCAGGAGCCGCTGGTCCGTGTCTTGGTTAATGATCGCGTTGTCCCGCTGC      1300
  Q  E  P  L  V  R  V  L  V  N  D  R  V  V  P  L
  '         400              '              410

ATGGGTGTCCGGTTGATGCTTTGGGGAGATGTACCCGGGATAGCTTTGTG
H  G  C  P  V  D  A  L  G  R  C  T  R  D  S  F  V
            '           420              '

AGGGGGTTGAGCTTTGCTAGATCTGGGGGTGATTGGGCGGAGTGTTTTGC      1400
 R  G  L  S  F  A  R  S  G  G  D  W  A  E  C  F  A
  430               '              440

TTAG                                                    1404
```

FIG.8B

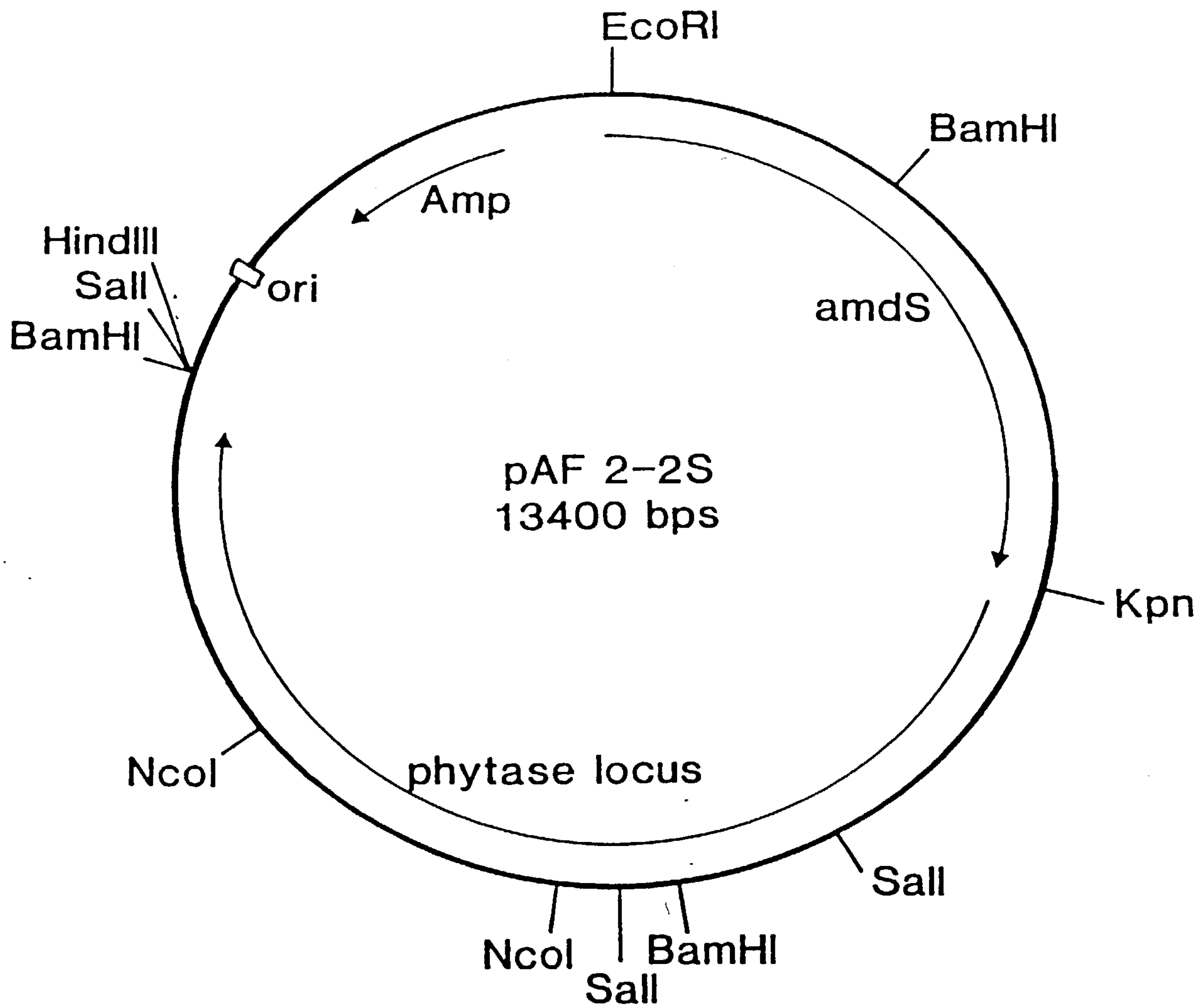
EcoRI
BamHI
Amp
HindIII
SalI
BamHI
ori
amdS
pAF 2-2S
13400 bps
KpnI
NcoI
phytase locus
SalI
NcoI
SalI
BamHI

FIG.9

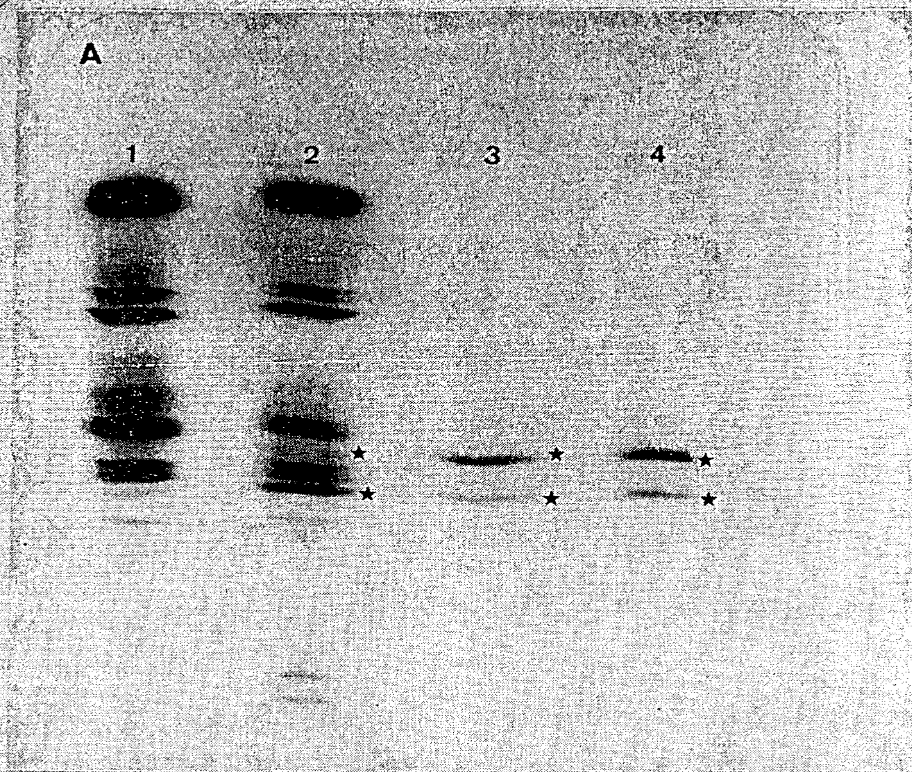

Figure 10A

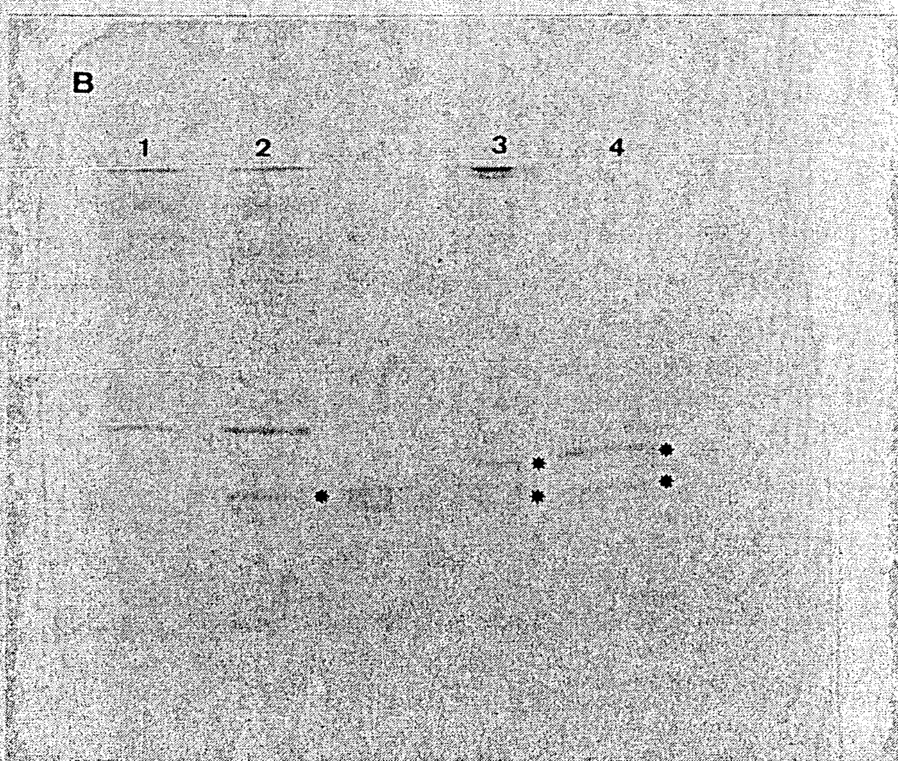

Figure 10B

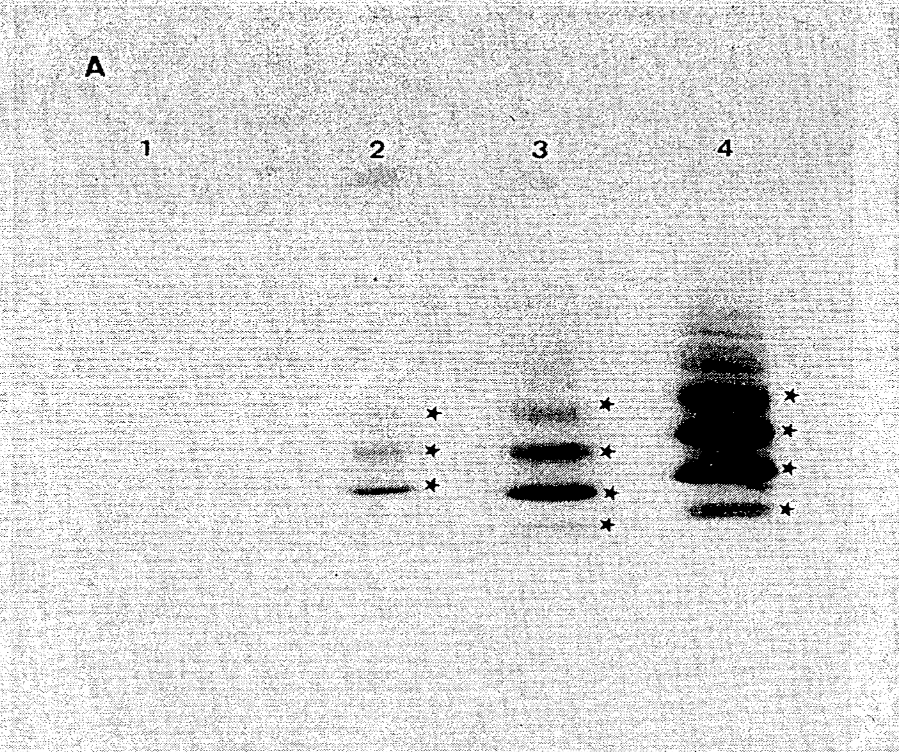

Figure 11A

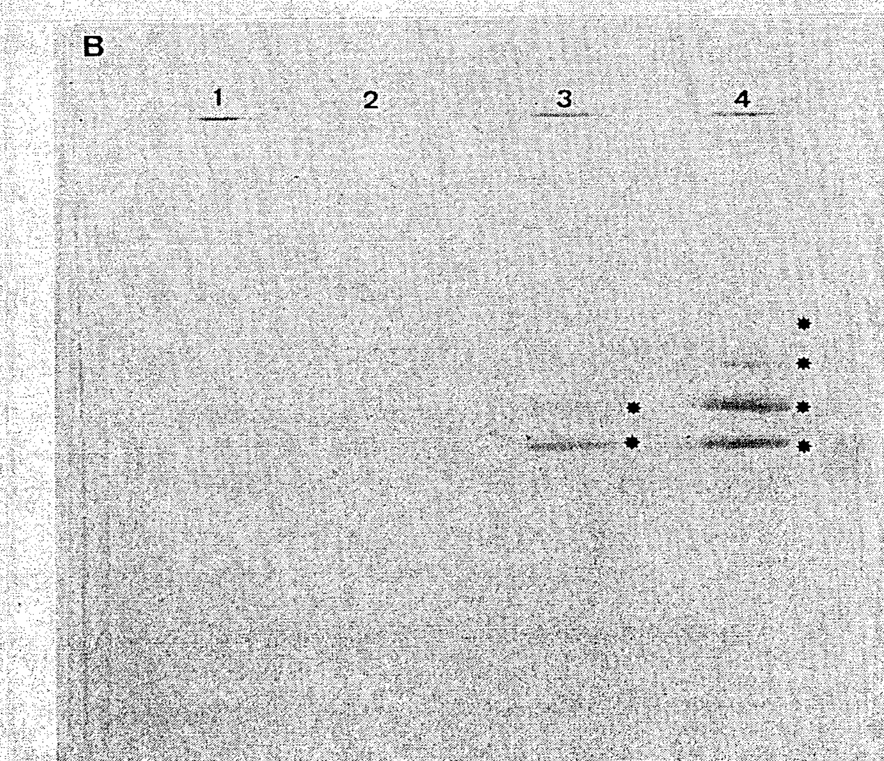

Figure 11B

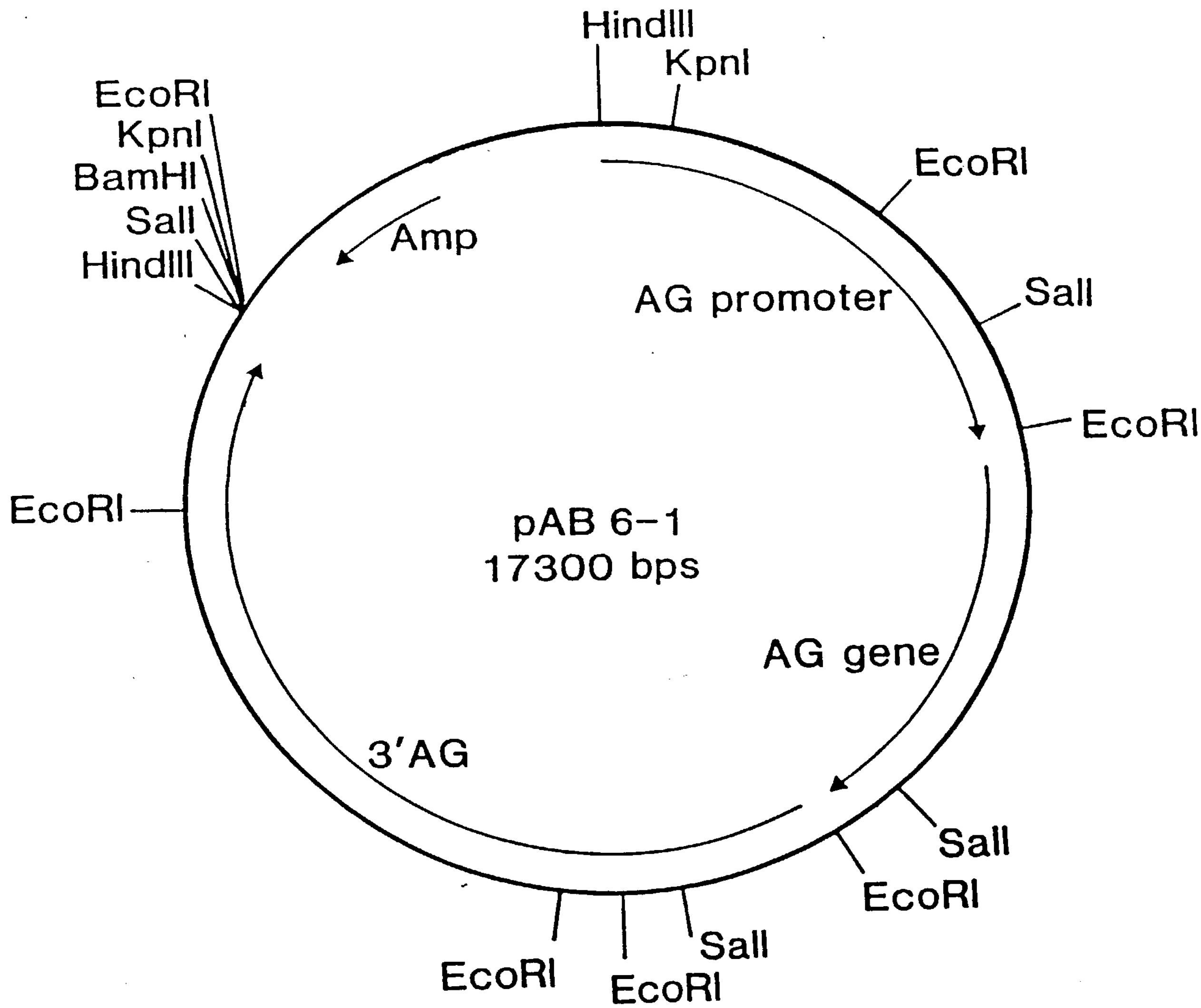
HindIII
KpnI
EcoRI
KpnI
BamHI
SalI
HindIII
Amp
EcoRI
AG promoter
SalI
EcoRI
EcoRI
pAB 6-1
17300 bps
AG gene
3'AG
SalI
EcoRI
SalI
EcoRI
EcoRI

FIG. 12

AG/PHYTASE GENE FUSIONS BY PCR

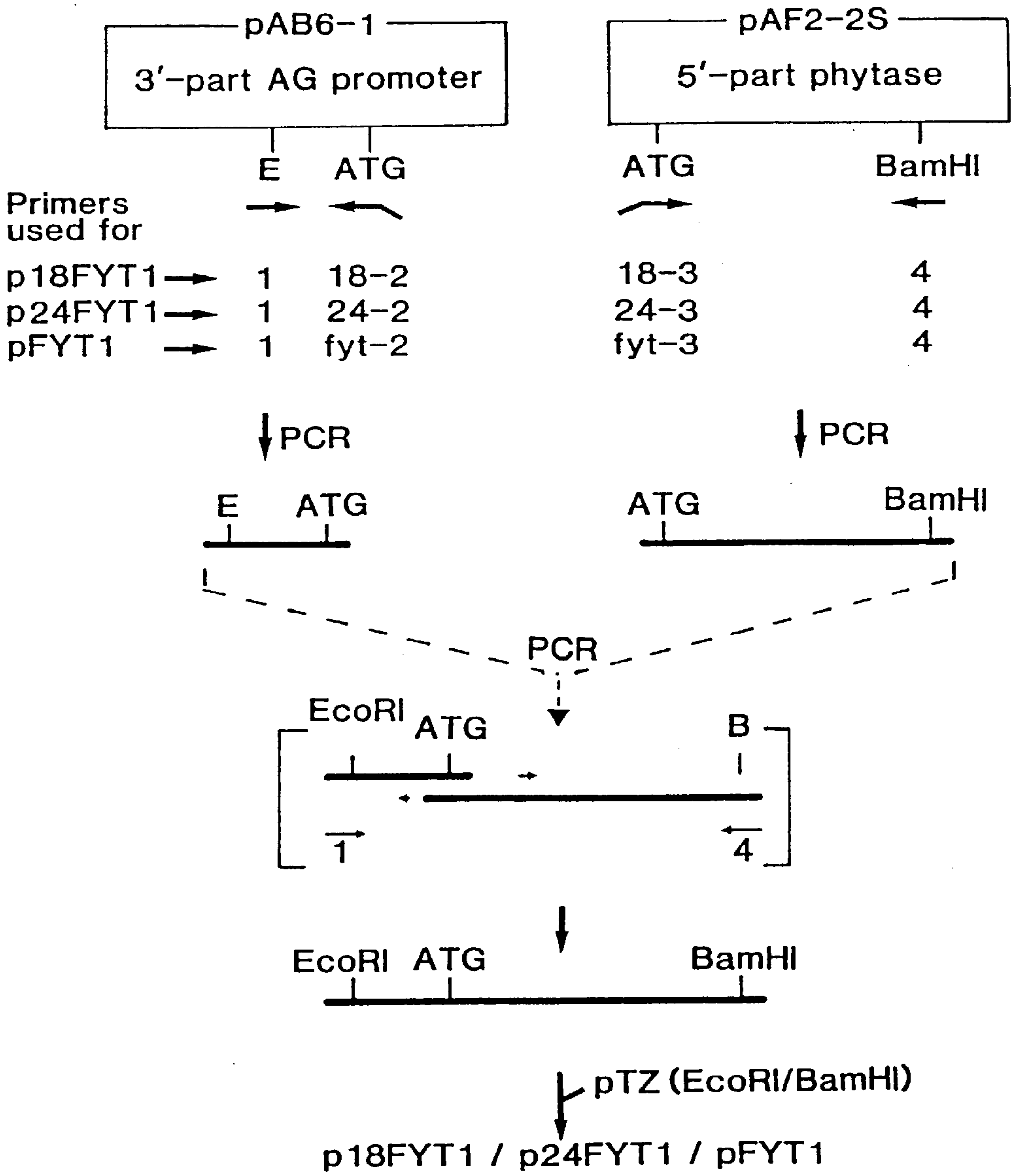

FIG. 13

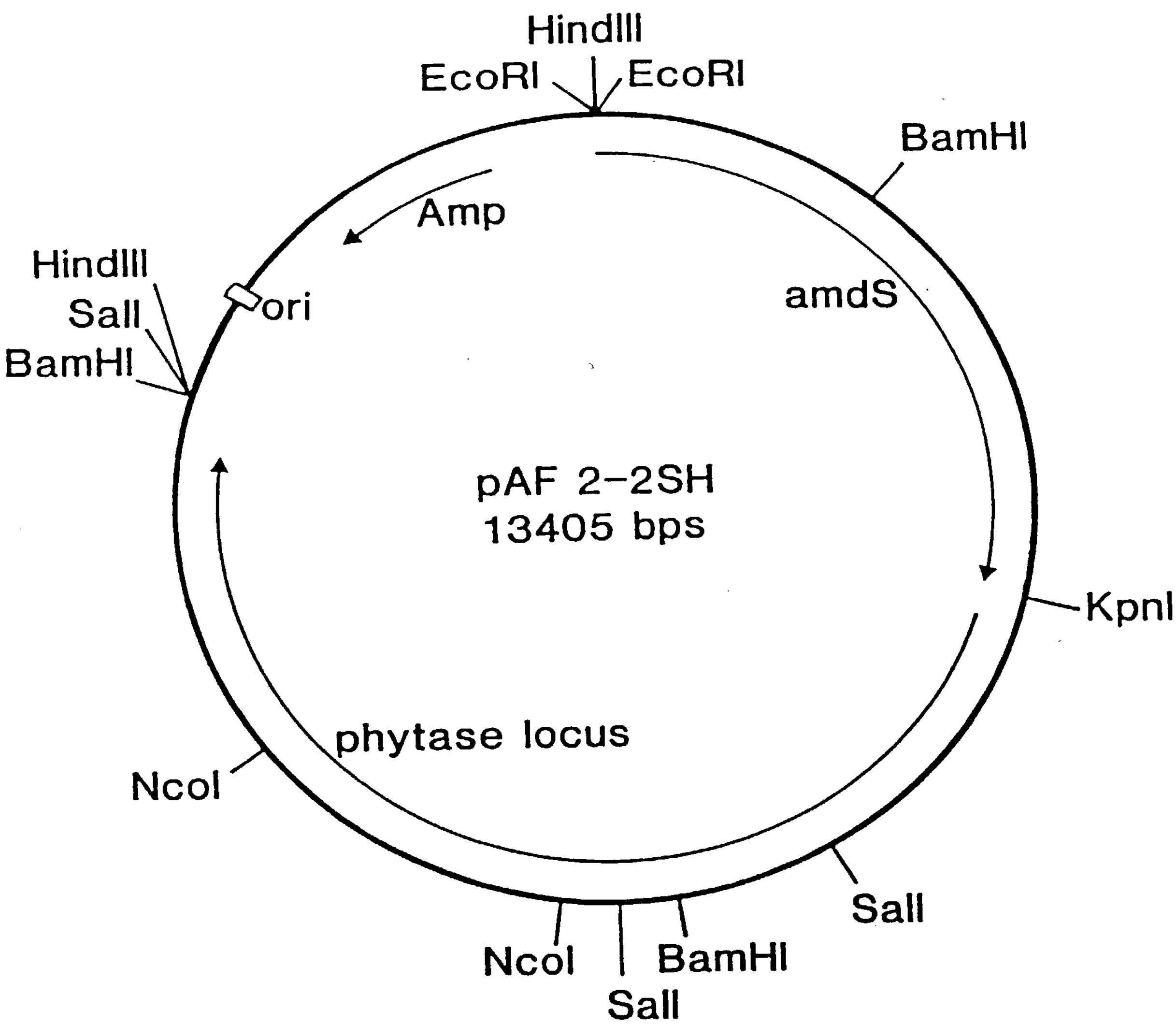
HindIII
EcoRI
EcoRI
BamHI
Amp
amdS
HindIII
SalI
BamHI
ori
pAF 2-2SH
13405 bps
KpnI
phytase locus
NcoI
SalI
NcoI
BamHI
SalI

FIG. 14

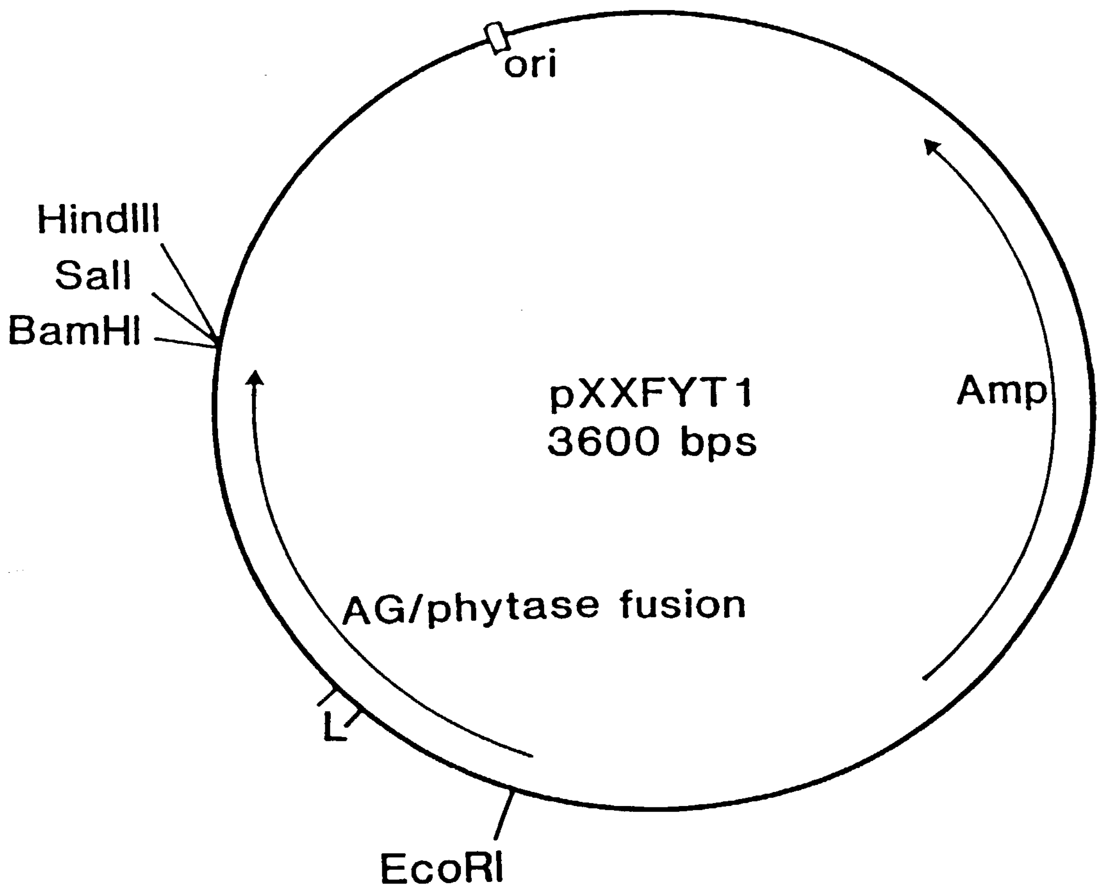
ori
HindIII
SalI
BamHI
pXXFYT1
3600 bps
Amp
AG/phytase fusion
L
EcoRI

FIG.15A

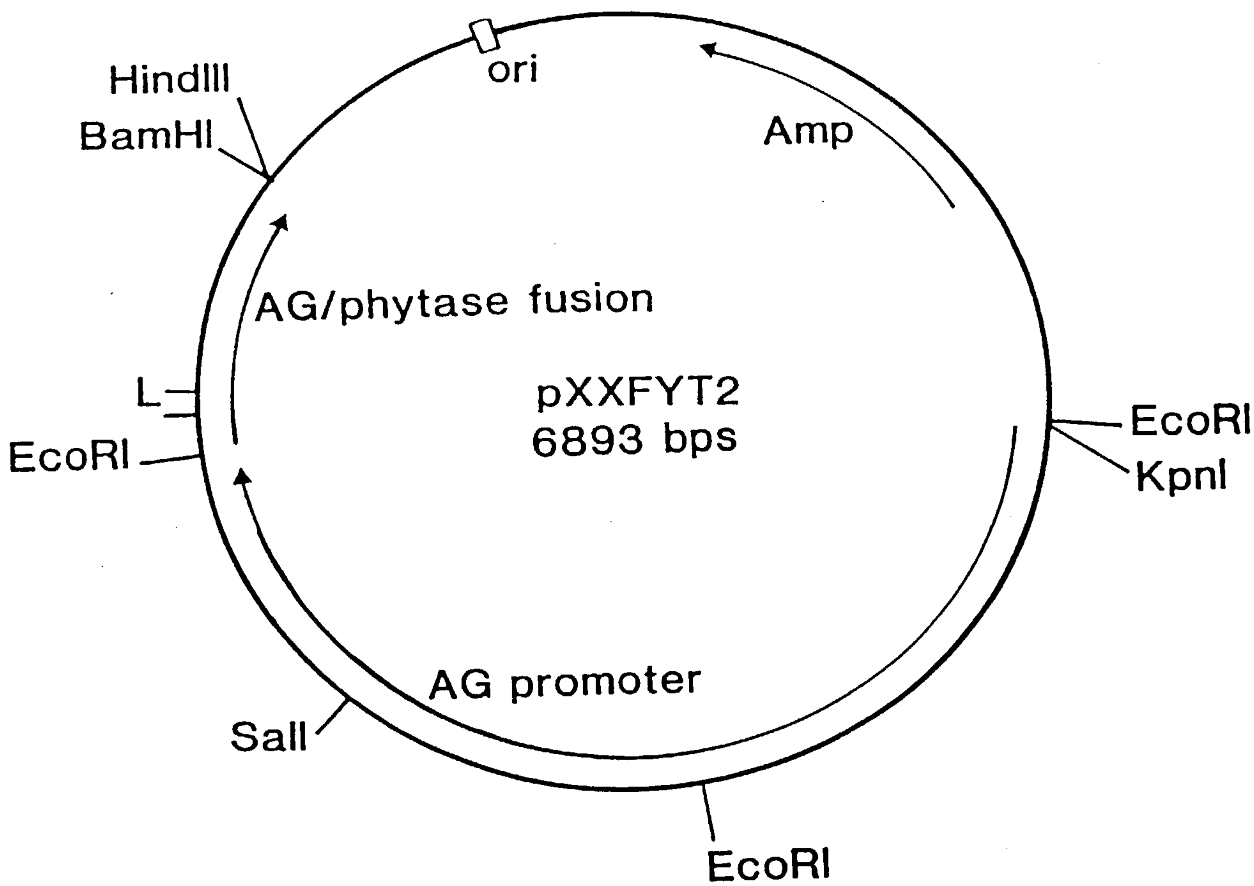
ori
Amp
HindIII
BamHI
AG/phytase fusion
pXXFYT2
6893 bps
L
EcoRI
EcoRI
KpnI
AG promoter
SalI
EcoRI

FIG. 15B

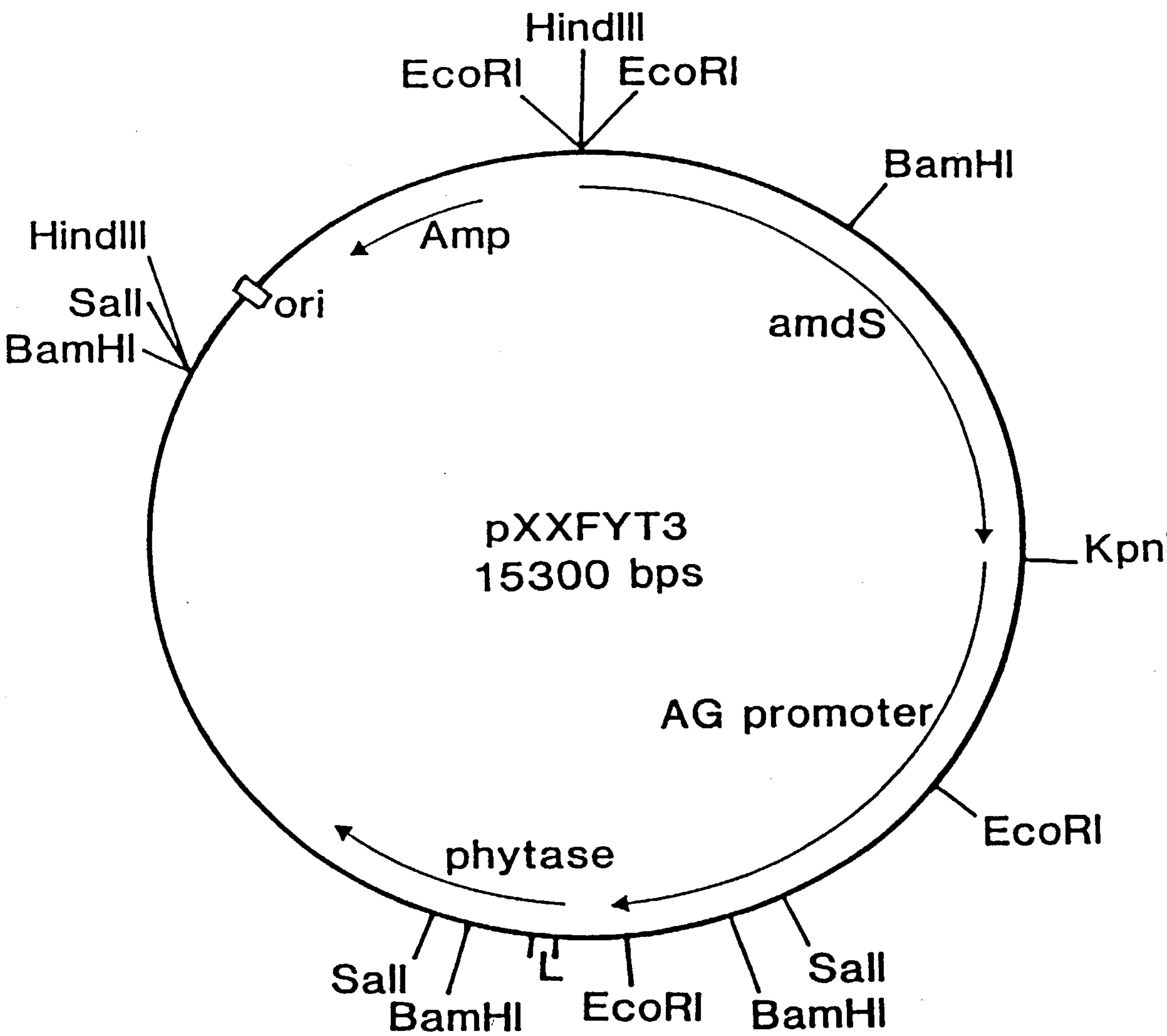
HindIII
EcoRI
EcoRI
BamHI
HindIII
SalI
BamHI
Amp
ori
amdS
pXXFYT3
15300 bps
KpnI
AG promoter
EcoRI
phytase
L
SalI
BamHI
EcoRI
SalI
BamHI

FIG. 15C

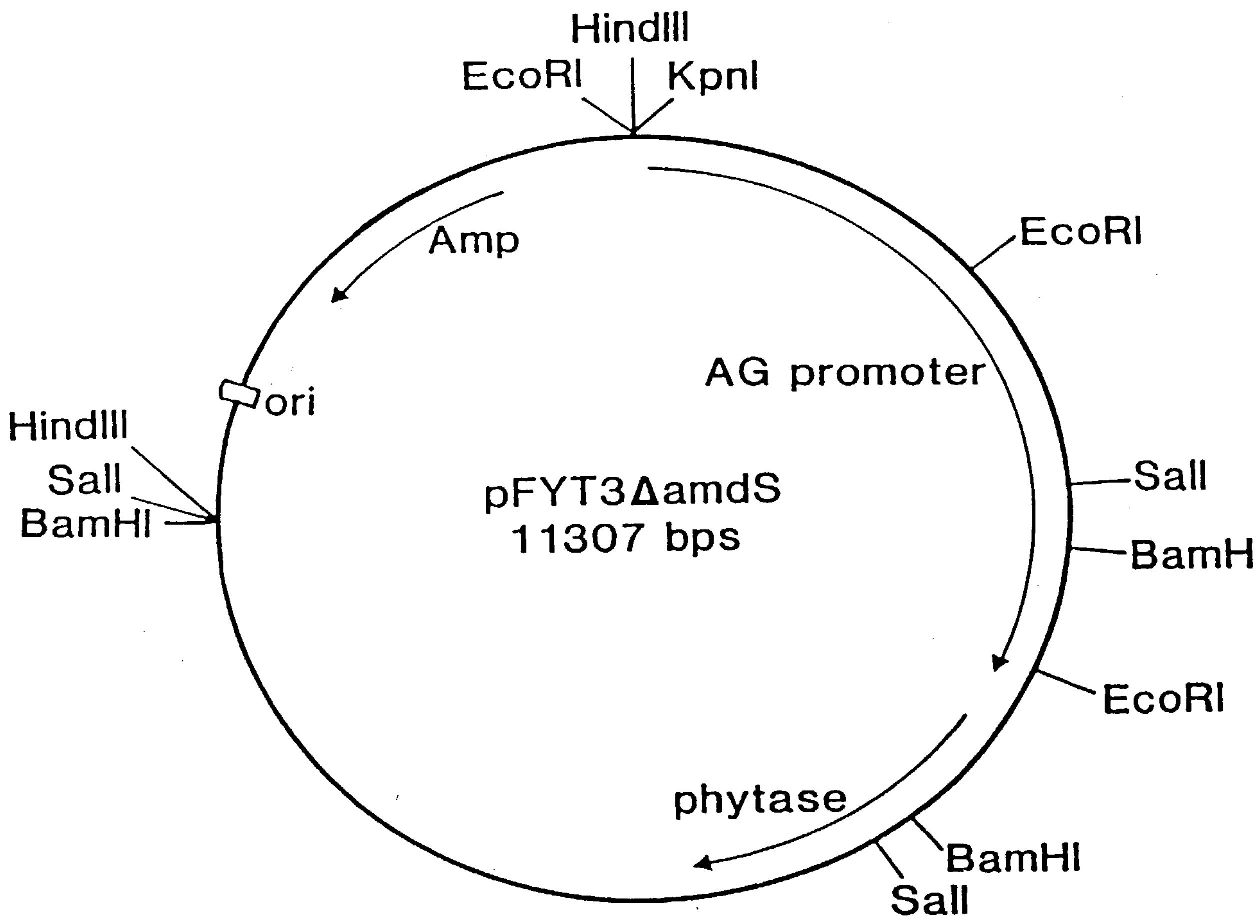
HindIII
EcoRI
KpnI
Amp
EcoRI
ori
AG promoter
HindIII
SalI
BamHI
pFYT3ΔamdS
11307 bps
SalI
BamHI
EcoRI
phytase
BamHI
SalI

FIG.16

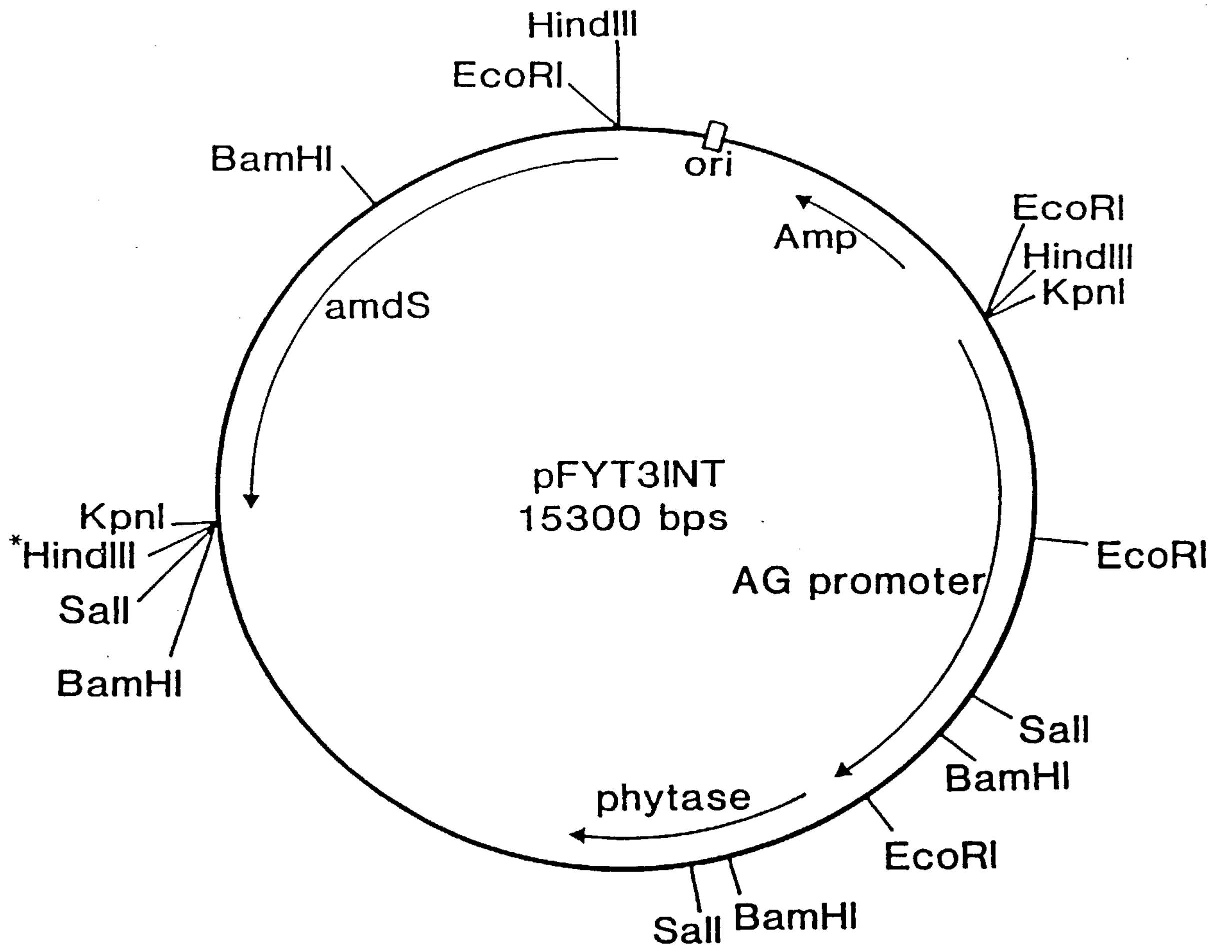
HindIII
EcoRI
BamHI
ori
Amp
EcoRI
HindIII
KpnI
amdS
pFYT3INT
15300 bps
KpnI
*HindIII
SalI
BamHI
EcoRI
AG promoter
SalI
BamHI
phytase
EcoRI
SalI
BamHI

FIG.17

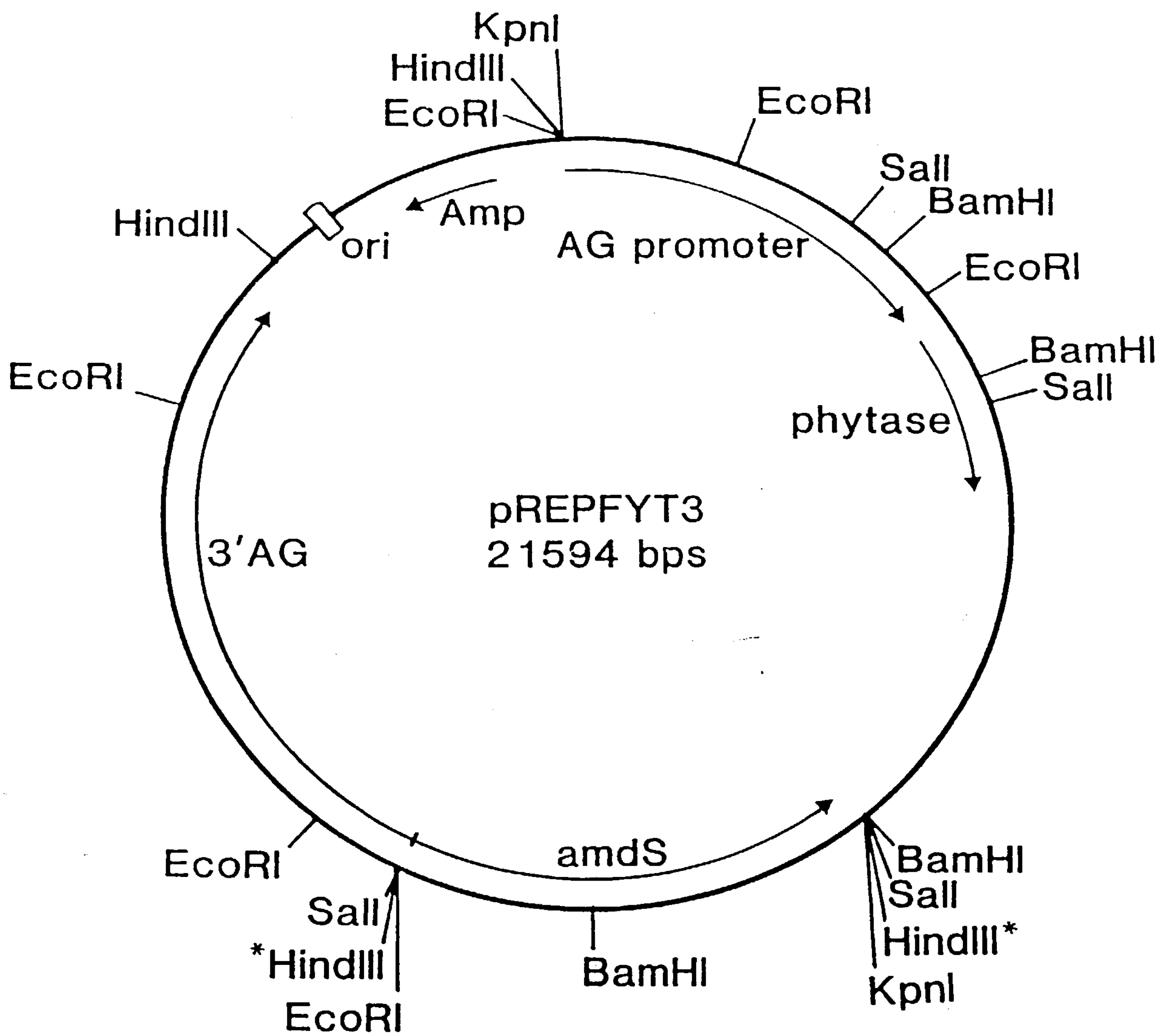
KpnI
HindIII
EcoRI
EcoRI
SalI
BamHI
EcoRI
BamHI
SalI
HindIII
ori
Amp
AG promoter
phytase
EcoRI
pREPFYT3
21594 bps
3'AG
amdS
EcoRI
SalI
*HindIII
EcoRI
BamHI
BamHI
SalI
HindIII*
KpnI

FIG. 18

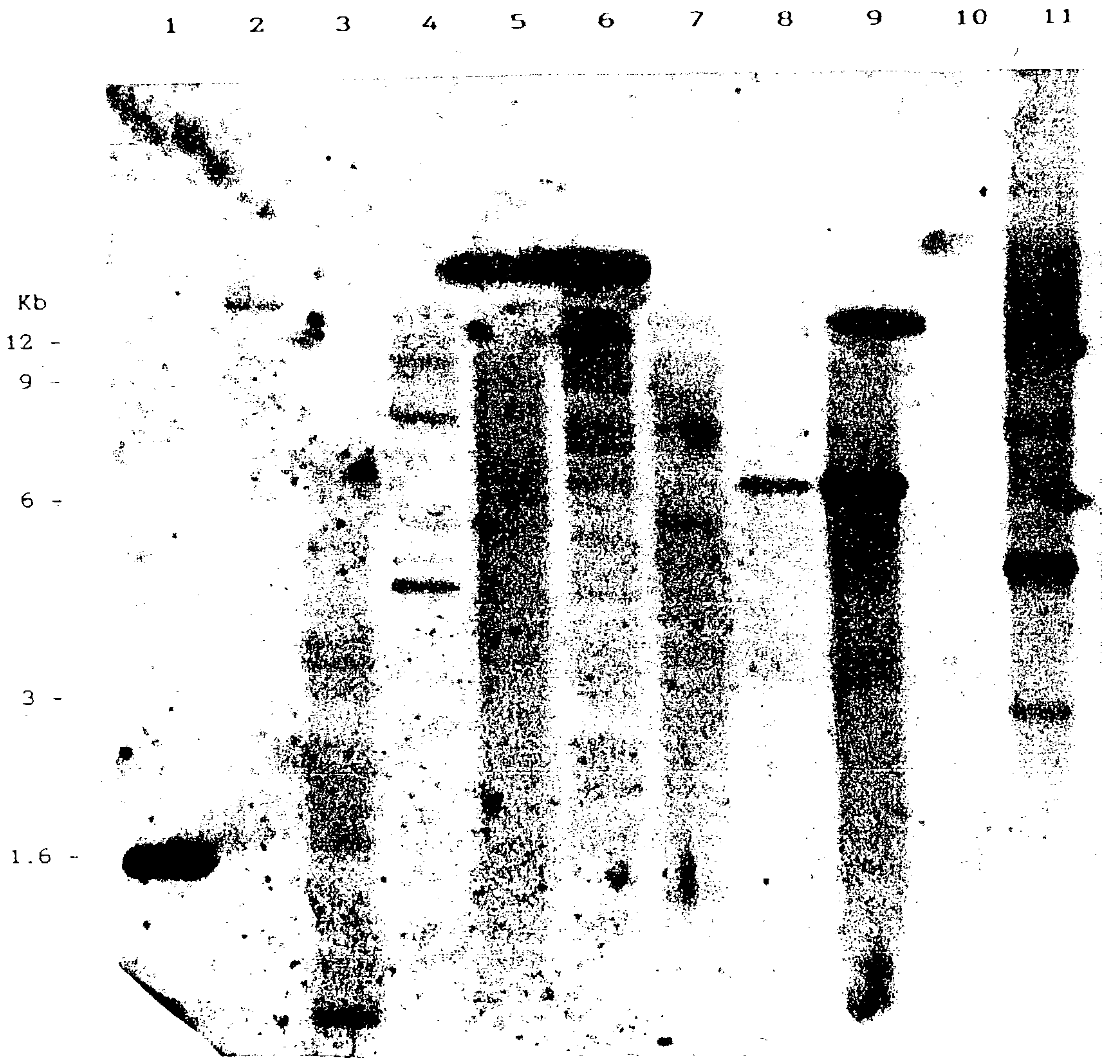

Figure 19A

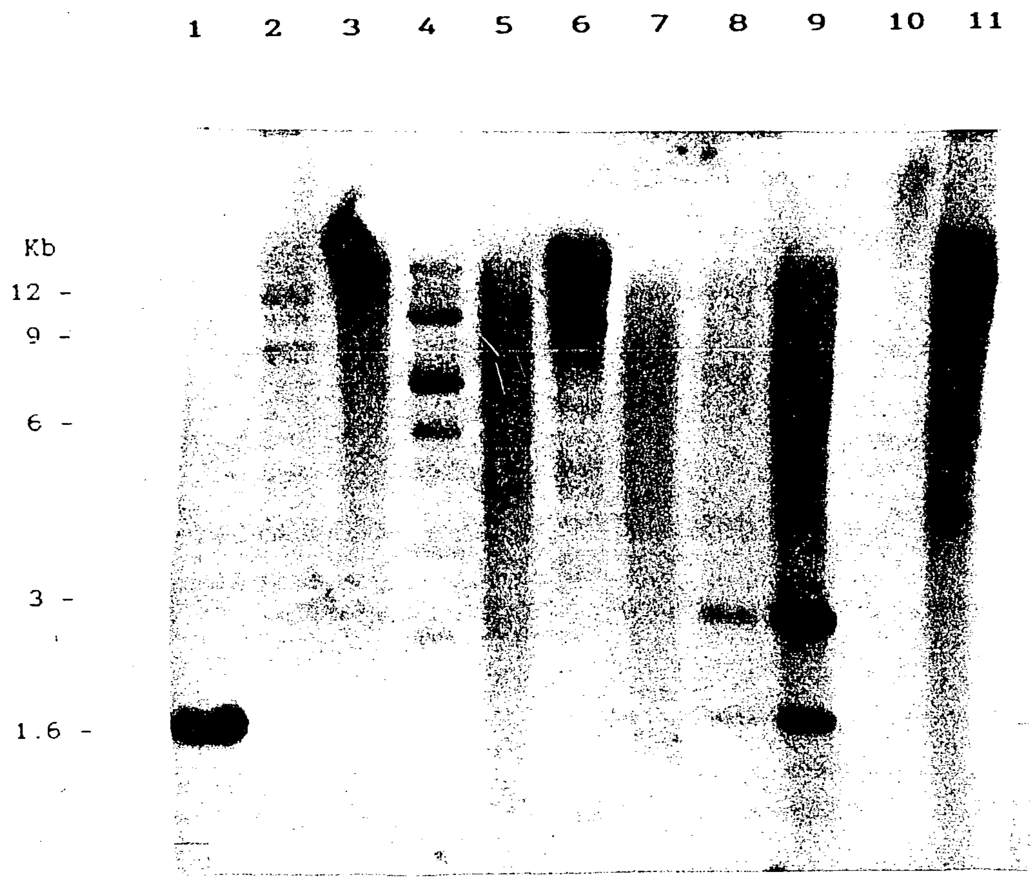

Figure 19B